Research Article

Antihyperlipidemic Activity of *Petroselinum crispum* Seeds in High Fat Diet-induced Atherosclerosis in Experimental Animals

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**Article history:**
Received: 24 October, 2021
Revised: 10 February, 2022
Accepted: 22 February, 2022
Published: 30 March, 2022

**Keywords:**
High fat diet (HFD), Lipid profile, *Petroselinum crispum* seeds, Oxidative stress.

**DOI:**
10.25004/IJPSDR.2022.140207

**ABSTRACT**

Atherosclerosis is one of the major contributing factors for the mortality due to cardiovascular diseases. Although a number of therapeutic agents are available still the management of atherosclerosis is not completely possible due to associated side effects with these therapeutic agents. Due to this herbal drug are considered as alternative for the treatment of atherosclerosis. Among these herbal drugs, *Petroselinum crispum* seeds are considered as potential agent for the treatment of atherosclerosis. In the present study, hydroalcoholic extract and petroleum ether extract of *P. crispum* seeds was prepared and anti-atherosclerotic activity was evaluated in high fat diet induced atherosclerosis in rats. Qualitative and quantitative analysis of hydroalcoholic extract was carried in vitro and in vivo antioxidant activity was evaluated. Anti-atherosclerotic activity was evaluated by measuring serum cholesterol, serum triglycerides, serum low-density lipoprotein (LDL), very low-density lipoprotein (VLDL) and high-density lipoprotein (HDL) level in animals. Animals were kept on high fat diet for 45 days and different doses of hydroalcoholic and petroleum ether extract were administered. Administration of different doses significantly attenuated the elevated level of serum cholesterol, serum triglycerides, serum LDL, VLDL and elevated HDL level. Oxidative stress was also found to be significantly attenuated in vitro and in vivo models. From the study it can be concluded that hydroalcoholic and petroleum ether extract of *P. crispum* seeds significantly attenuated elevated oxidative stress and lipid profile.

**INTRODUCTION**

Cardiovascular diseases (CVDs) are the leading cause of morbidity and mortality in both developing and developed nations.[1] Almost 60% of deaths are due to CVD. Almost 41% death in South Africa, 35% in India, 28% in Brazil, 12% in USA and 9% in Portugal between the ages of 35-44 years were due to CVD.[2] The incidence of CVD events was 3 times higher in atherosclerotic patients compared with individuals without atherosclerosis. Atherosclerosis is leading cause of death and responsible for 75% of cardiovascular-related deaths. Increase in lipid level is the major contributor responsible for 77% increased coronary artery disease (CAD) mortality. The major risk factors for the CVD are hyperlipidemia, smoking, hypertension, type 2 diabetes (T2D), high blood pressure, physical inactivity and obesity.[3] Smoking is estimated to cause nearly 10% of the CVD. Cardiovascular risk increases with increase in the levels of glucose.[4] Out of 17.1 million populations suffering from CVD, from which 1-million people suffer from atherosclerotic plaque. The pharmacotherapy of atherosclerosis is for life long. Many lipid lowering drugs are available for the treatment of atherosclerosis, e.g., statins (inhibits HMG-CoA reductase causes reduction in LDL levels),[5] bile-acid sequestrants (production of LDL receptors causes more clearance and lowers the level of LDL),[6] niacin (inhibits lipolysis of triglycerides by hormone-sensitive lipase),[7] PPAR activators (fibracic acid derivatives), ezetimibe, etc. But these allopathic drugs have various side effects like statins are responsible for elevation of hepatic transaminase and myopathy,[8] bile acid sequestrants causes hypercholestermic acidosis[9] and niacin causes fulminant hepatic failure whereas PPAR...
Hypolipidemic Activity of Parsley

activators causes gallstone and biliary tract disease.\textsuperscript{[10]} Most of these drugs have serious side effects which lead to discontinuation of the pharmacotherapy. Therefore, to overcome these side effects, traditional medicinal plants are being explored for safer and specific management of atherosclerosis. The use of herbal medicines is very popular in all over the world. People use herbal products to maintain good health. Parsley is a culinary herb originated from the Mediterranean region belonging to family Apiaceae.\textsuperscript{[11]} It is widely cultivated as an herb, spice and vegetable. Raw parsley contains carbohydrates, sugar, dietary fibers, fat, protein, vitamins, calcium, iron, magnesium, phosphorus, potassium and zinc (USDA Nutrient Database). So, the present study was designed to explore the anti-atherosclerotic property of \textit{Petroselinum crispum} (Parsley) in rats using high fat diet (HFD) induced atherosclerosis.\textsuperscript{[12]}

\textbf{Experimental Animals}

Wistar rats (150–180g), obtained from the Paradise rabbit farm, Jind, Haryana and transported to the animal house at Bilwal Medchem and Research Laboratory Pvt. Ltd., Jaipur, Rajasthan, India. The use of these animals and the study protocols were approved by Institutional Animal Ethics Committee (IAEC) against reg. no. 2005/PO/RcBT/S/18/ CPCSEA. The animals were facilitated with environmental conditions of photoperiod (12:12 h dark: light cycle) and temperature (25 ± 2°C) and provided with commercial diet and water \textit{ad libitum}.

\textbf{Identification and Authentication of Plant Material}

Seeds of plant were collected from the local market, Rohtak, Haryana, India. The seeds of \textit{P. crispum} was identified and authenticated from Department of Botany, MDU, Rohtak, Haryana, India.

\textbf{Material and Method}

\textbf{Instruments}

Digital balance, Centrifuge, Semi-analyser, B.O.D incubator, Sonicator, Total cholesterol estimation kit, Triglyceride kit, HDL kit, UV spectrophotometer and rotary evaporator.

\textbf{Chemicals and Reagents Used}

Fenofibrate (50mg/kg), Petroleum ether, Hydroethanol (40%), Ascorbic acid, gallic acid, Folin-Ciocalteau reagent, Trichloroacetic acid, 2,2-Diphenyl-1-picrylhydrazyl (DPPH), Thiobarbituric acid, Disodium hydrogen phosphate, Glutathione and DTNB [5, 5’-Dithiobis (2-nitrobenzoic acid)].

\textbf{Selection and Preparation of Different Doses}

Petroleum ether and hydroalcoholic extracts of the plant were prepared in suspension form in distilled water. The strength of the suspension varies with the dose formulated and was expressed as w/v of dried powdered seeds. Equal portion from each fraction of plants powder was subjected at 60 to 70°C using mixture of ethanol and water (60:40) in a soxhelt apparatus and then filtered using Whatman no.1 filter paper. The filtrate was evaporated at 55°C in hot air oven. Fenofibrate 50 mg/kg was taken as the reference standard drug for evaluation of antihyperlipidemic activity and the same was also formulated as suspension in distilled water.\textsuperscript{[13]} Different doses of the extract was selected and administered; 250, 500, and 1000 mg/kg for both the extracts and the inhibition of lipid peroxidation along with their effects on other factors like body weight, total serum cholesterol, serum HDL and serum LDL etc. was found out.

\textbf{Acute Oral Toxicity Study}

The acute oral toxicity studies for the extracts were carried as per the OECD guidelines 423.\textsuperscript{[14]} Each animal was administered with the hydroalcoholic extract of the herbal formulation by oral route and the animals were kept under observation for mortality up to 72 hours. The hydroalcoholic extract of herb was found to be safe up to 1000 mg/kg body weight.

\textbf{Antioxidant Activity}

\textit{In-vitro Antioxidant Assay}

\textbf{Estimation of Total Phenolic Compounds}

Total soluble phenolics in the extracts were determined with Folin-Ciocalteau reagent according to the method using gallic acid as a standard phenolic compound 1.0 mL of extract solution containing 1.0 g extract in a volumetric flask was diluted with 45 mL of methanol. 1.0 mL of Folin-Ciocalteau reagent was added and mixed thoroughly. Three minutes later 3.0 mL of 2% sodium carbonate was added and the mixture was allowed to stand for 3 hours with intermittent shaking. The absorbance of the blue colour that developed was read at 760 nm. The concentration of total phenols was expressed as mg/g of dry extract. All determinations were performed in triplicate. Total content of phenolic compounds in plant extract was determined as μg of gallic acid equivalents (GAE).\textsuperscript{[15]}

\textbf{Determination of DPPH (1-1-diphenyl 2-picryl hydrazyl) Radical Scavenging Activity}

The free radical-scavenging activity of the hydroalcoholic extract was observed in terms of hydrogen donating or radical-scavenging ability using the stabler radical DPPH. 0.1 mM solution of DPPH in ethanol was prepared and 1.0 mL of this solution was added to 3.0 mL of extract solution in water at different concentrations (10–320 μg/mL). Thirty minutes later, the absorbance was observed at 517 nm. Ascorbic acid was used as the reference compound. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity.\textsuperscript{[16]} Radical-scavenging activity was expressed as the inhibition percentage of free radical.
by the sample and was calculated using the following formula:

\[
\%\text{ inhibition} = \left( A_0 - A_i \right) / A_0 \times 100;
\]

Where \( A_0 \) was the absorbance of the control (blank, without extract) and \( A_i \) was the absorbance in the presence of the extract. All the tests were performed in triplicate and the graph was plotted with the mean values.

**In vivo Antioxidant Assay**

**Estimation of Lipid Peroxidation**

The quantitative measurement of thiobarbituric acid reactive substances (TBARS), an index of lipid peroxidation was performed. 0.2 mL of supernatant of homogenate of the extract was pipetted out in a test tube, followed by addition of 0.2 mL of 8.1% sodium dodecyl sulphate, 1.5 mL of 30% acetic acid (pH 3.5), 1.5 mL of 0.8% of thiobarbituric acid and the volume was made up to 4 mL with distilled water. The test tubes were incubated for 1 hr at 95°C, then cooled and added 1 mL of distilled water followed by addition of 5 mL of n-butanol-pyridine mixture (15:1 v/v). The tubes were then centrifuged at 4000 rpm for 10 minutes. The absorbance of developed pink colour was measured spectrophotometrically at 532 nm. A standard calibration curve was prepared using 1-10 nM of 1,1,3,3-tetramethoxy propane. The Thiobarbituric acid reactive substances (TBARS) values were expressed as nanomoles per mg of protein.17

**Estimation of Reduced Glutathione**

The reduced glutathione (GSH) content in tissue was estimated using nitroprusside reaction for glutathione. The supernatant of extract was mixed with trichloroacetic acid (10% w/v) in 1:1 ratio. The tubes were centrifuged at 4000 rpm for 10 minutes. The supernatant of extract was mixed with trichloroacetic acid (10% w/v) in 1:1 ratio. The tubes were centrifuged at 4000 rpm for 10 minutes. The supernatant obtained (0.5 mL) was mixed with 2 mL of 0.3 M disodium hydrogen phosphate. Then 0.25 mL of 0.001 M freshly prepared DTNB [5,5’-dithiobis (2-nitrobenzoic acid) dissolved in 1% w/v citric acid] was added and absorbance was observed spectrophotometrically at 412 nm.18 A standard curve was plotted using 5 to 50 μM of reduced form of glutathione and results were expressed as micromoles of reduced glutathione per mg of protein.

**Anti-atherosclerotic Activity**

**HFD Induced Atherosclerosis**

Atherosclerosis was induced by high-fat diet (30% fat by weight), which contained the same vitamin and mineral content as the control diet but was high in fat, coconut oil, sugar (sucrose 30%) and gram flour. The high fat diet comprises of 2% cholesterol, 1% cholic acid in 1 mL coconut oil along with 20% butter oil by oral gavage for 15 days.

The animals (150–175 g) were used for the study and divided in nine groups for 45 experimental periods. Each group comprised of six animals. Group I was kept on standard laboratory diet and water *ad libitum*, Group II kept on high fat diet. Group III was standard group kept on high fat diet and treated with fenofibrate (50 mg/kg).18 Group IV, V, VI were test groups kept on high fat diet and treated with different doses of petroleum ether extract; Group VII, VIII and IX were test groups kept on high fat diet and treated with different doses of hydroalcoholic extract. The animals were kept on fasting overnight for blood withdrawal.

Blood withdrawal was done by retro orbital puncture. Different animals groups in the experimental protocol are given in the Table 1.

**Table 1: Experimental protocol**

<table>
<thead>
<tr>
<th>Group I: (Normal Control)</th>
<th>On laboratory diet and water <em>ad libitum</em> for 45 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group II: (Atherogenic Control)</td>
<td>On high fat diet for 45 days</td>
</tr>
<tr>
<td>Group III: Treatment group (HFD+Fenofibrate)</td>
<td>High fat diet for 45 days +Fenofibrate (50 mg/kg, <em>per oral</em>) after 15 days &amp; continued upto 45 days</td>
</tr>
<tr>
<td>Group IV: Treatment group (HFD + Pet Ether extract 250 mg/kg)</td>
<td>High fat diet +pet ether extract (250 mg/kg, <em>per oral</em>) after 15 days and continued up to 45 days</td>
</tr>
<tr>
<td>Group V: Treatment group (HFD + Pet Ether extract 500 mg/kg)</td>
<td>High fat diet + pet ether extract (500 mg/kg, <em>per oral</em>) was continued after 30 days</td>
</tr>
<tr>
<td>Group VI: Treatment group (HFD + Pet Ether extract 1000 mg/kg)</td>
<td>High fat diet + pet ether extract (1000 mg/kg, <em>per oral</em>) was continued after 30 days</td>
</tr>
<tr>
<td>Group VII: Treatment group (HFD + hydroalcoholic extract 250 mg/kg)</td>
<td>High fat diet + hydroalcoholic extract (250 mg/kg, <em>per oral</em>) was continued after 30 days</td>
</tr>
<tr>
<td>Group VIII: Treatment group (HFD + hydroalcoholic extract 500 mg/kg)</td>
<td>High fat diet + hydroalcoholic extract (500 mg/kg, <em>per oral</em>) was continued after 30 days</td>
</tr>
<tr>
<td>Group IX: Treatment group (HFD + hydroalcoholic extract 1000 mg/kg)</td>
<td>High fat diet + hydroalcoholic extract (1000 mg/kg, <em>per oral</em>) was continued after 30 days</td>
</tr>
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The results of phytochemical screening of petroleum ether and hydroalcoholic extracts (ethanol and water in 60:40) of *P. crispum* are given in Table 2.

The phytochemical screening indicates that phenols, tannins, flavanoids, glycosides and triterpenoids are present in petroleum ether extract, while flavanoids,
triterpenoids, phenols, tannins, saponins and amino acids are present in hydroalcoholic extract of *P. crispum* seeds.

**Quantitative Phytochemical Analysis**

**Flavanoids**

For determination of flavonoid content in the sample, aluminium chloride colorimetric method was followed. Quercetin was employed for making the standard calibration plot. The sample was observed spectrophotometrically, and the flavonoid content was calculated and expressed as quercetin equivalent per g of dried sample. The amount of flavanoids was found to be 12.35 mg quercetin acid equivalent (QE)/g dry weight.

**Terpenoids**

Powder of seeds of *P. crispum* (10 g) was soaked for about 24 hours. This was then filtered and extracted with petroleum ether. The collected ether extract was then dried and the % of terpenoid content was calculated as:

% content = (Weight of terpenoid extract / weight of the sample) x 100

The amount of terpenoids was found to be 0.47 g

**Saponins**

Saponins was analysed quantitatively by the method reported by Ejikeme et al. and Obadoni and Ochuko. The saponin content was calculated as a percentage:

% Saponin = Weight of saponin / Weight of sample x 100

The amount of saponins was found to be 0.59 g.

**In vivo Antioxidant Activity**

**Effect of Hydroalcoholic Extract and Petroselinum Extract of *P. crispum* Seeds on Lipid Peroxidation in HFD Model**

The hydroalcoholic and petroleum ether extract significantly decreased the lipid peroxidation in serum at different doses (250, 500, and 1000 mg/kg). The inhibition of lipid peroxidation according to dose was found to be PH 250 mg/kg (1.85 nM MDA/mg protein), PP 250 mg/kg (1.84 nM MDA/mg protein), PH 500 mg/kg (1.35 nM MDA/mg protein), PP 500 mg/kg (1.58 nM MDA/mg protein); PH 1000 mg/kg (1.13 nM MDA/mg protein), PP 1000 mg/kg (1.22 nM MDA/mg protein).

*Estimation of reduced GSH in HFD induced atherosclerotic rats*

The hydroalcoholic and petroleum ether extract statistically increased the GSH levels at various doses PH 250 mg/kg (43.47 μM/mg protein), PP 250 mg/kg (41.28 μM/mg protein); PH 500 mg/kg (52.79 μM/mg protein), PP 500 mg/kg (50.13 μM/mg protein); PH 1000 mg/kg (61.13 μM/mg protein), PP 1000 mg/kg (55.65 μM/mg protein) at 45th day as compared to atherosclerotic control (41.97 μM/mg protein) in high fat diet model.

**Anti-atherosclerotic Activity in HFD Induced Atherosclerosis**

**Effect of Petroleum Ether and Hydroalcoholic Extract on Body Weight in High Fat Diet Induced Atherosclerosis in Rats**

Administration of high fat diet for 45 days resulted in significant weight gain in rats. Whereas administration of different doses of both the extracts; hydroalcoholic and petroleum ether (250, 500, and 1000 mg/kg) produced significant attenuation in body weight as compared to
control group. The petroleum ether extract dose 500 mg/kg produced significant decrease in body weight as compared to dose 250 mg/kg. The hydroalcoholic extract at dose 500 mg/kg produced more significant results than 250 and 1000 mg/kg produced significant decrease in body weight as compared to 500 mg/kg.

Effect of Petroleum Ether and Hydroalcoholic Extract on Total Serum Cholesterol in High Fat Diet induced Atherosclerosis in Rats

Administration of high fat diet for 45 days significantly elevated the serum cholesterol levels. But administration of different doses of both the extracts; petroleum ether and hydroalcoholic extract (250, 500, and 1000 mg/kg) produced significantly attenuation in cholesterol level as compared to atherogenic control. The petroleum ether extract dose at 250 mg/kg produced significantly decrease cholesterol levels as compared to atherogenic control. The hydroalcoholic extract dose 500 mg/kg produced significantly decrease cholesterol levels as compared to 250 mg/kg while 1000 mg/kg produced significantly attenuates cholesterol levels as compared to 500 mg/kg.

Effect of Petroleum Ether and Hydroalcoholic Extract on Serum HDL in High Fat Diet induced Atherosclerosis in Rats

High fat diet was administered for 45 days which significantly attenuates the levels of HDL. Petroleum ether and hydroalcoholic extracts when administered at different doses produced significantly increase the levels of HDL as compared to atherogenic control. The petroleum ether extract and hydroalcoholic extract dose 250 mg/kg produced significantly increases the levels of HDL as compared to atherogenic control.

Effect of Petroleum Ether and Hydroalcoholic Extract on Serum LDL in High Fat Diet induced Atherosclerosis in Rats

Administration of HFD for 45 days produced significantly increases the serum LDL levels. While administration of different doses of extract petroleum ether (250 mg/kg, 500 mg/kg and 1000 mg/kg) produced significant attenuation of LDL levels as compared to atherogenic control. The dose 250 mg/kg of petroleum ether extract produced significantly elevated the levels of LDL as compared to atherogenic control and 1000 mg/kg produced decrease the levels of LDL as compared to 500 mg/kg. The 250 mg/kg hydroalcoholic extract produced significantly attenuation in the LDL levels as compared to atherogenic control. 500 mg/kg produced statistically significant decrease the levels of LDL as compared to 250 mg/kg.

Effect of Petroleum Ether and Hydroalcoholic Extract on Serum VLDL in High Fat Diet induced Atherosclerosis in Rats

When high fat diet was administered for 45 days, it causes significantly attenuation in the VLDL levels; whereas the administration of different doses of petroleum ether and hydroalcoholic extracts statistically causes a significant decrease in the VLDL levels as compared to atherogenic control. The petroleum ether extract at dose 250 mg/kg produced significantly decreases the levels of VLDL as compared to atherogenic control. In the hydroalcoholic extract 250 mg/kg produced significant decrease the levels of VLDL as compared to atherogenic control while 500 mg/kg produced more significant decreases the levels as compared to 250 mg/kg.
glyceride as compared to atherogenic control. While the hydroalcoholic extract at dose 500 mg/kg produced significant decrease the level of triglyceride as compared to dose 250 mg/kg.

**Discussion**

Atherosclerosis is a chronic inflammatory in which fatty material is deposited in the central core of arteries leading to the formation of plaque covered with fibrous cap.[20] Hyperlipidemia has been documented as one of the major causative factor for atherosclerosis, resulting in coronary heart diseases (CHD).[21] Elevated cholesterol particularly LDL are the major reasons attributed to cardiovascular diseases. The imbalance in the lipid metabolism plays a role in aggravating the lipid peroxidation. Hyperlipidemia increases lipid peroxidation and decrease reduced glutathione which enhances oxidative stress and damage on the endothelial wall and SMCs.[21]

Phytochemical evaluation of PH and PP extracts of *P. crispum* seeds revealed the presence of flavonoids, saponins, tannins and glycosides. Flavonoids like luteolin, apigin and apigenin which acts as antioxidant and protects the arteries from oxidative stress and damage. Parsley contains chlorophyll which alkalizes the body and purifies the blood vessels. It helps to enhance the cellular glutathione formation which is used to detoxify and heals the body effectively.[21]

In the present study high fat diet model was employed to evaluate the anti-atherosclerotic activity. The high fat diet administered in present study produced significant elevation in hyperlipidemia. Fat-enriched diets have been used for decades to model obesity, dyslipidemia and insulin intolerance in rodents. It has been observed that the disorders achieved by high-fat feeding resemble the human metabolic syndrome closely, and this also may extend to the cardiovascular complications.[22] This was also found that the dietary unsaturated fats promote the accumulation of oleoyl-CoA (substrate for ACAT2) in the liver responsible for the accumulation of ACAT2- derived cholesteryl oleate. This cholesteryl oleate secretes into lipoproteins, thereby enhancing atherosclerosis. Hepatic ACAT2 gets esterified to cholesterol and secreted into plasma compartment. Plasma cholesteryl oleate enriched with LDL particles.[22] These LDL gets oxidized and deposited on the artery walls. The significant change in lipid profile noticed in the experimental animals confirmed the induction of hyperlipidemia in HFD fed rats. Animals were subjected to HFD for 45 days resulting in significant elevation in total cholesterol, LDL, VLDL, triglyceride levels whereas HDL level was found to be attenuated.[23] Phytochemical analysis revealed the presence of significant amount of β-carotene may be responsible for effects in the PH and
PP extracts. Beta-carotene, fat soluble antioxidant, inhibits lipid peroxidation leading to the inhibition of accumulation of oxidized low-density lipoprotein (oxLDL) on artery walls thus reduces cholesterol, LDL and triglyceride. Flavanoids were previously registered to contribute health promotion by diminishing the biosynthesis of cholesterol and enhance the phosphorylation of HMG-CoA reductase.[23] In vitro oxidative stress measured in terms of reducing power assay, 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging assay and in vivo oxidative stress measured in terms of lipid peroxidation and reduced glutathione levels. *P. crispum* seeds are rich in flavanoids like luteolin, apigen and apigenin, vitamin C and A which acts as antioxidant and protects the arteries from oxidative stress and damage.[23] In the present study similar results were observed. Administration of petroleum ether and hydroalcoholic extracts of *P. crispum* significantly attenuated the oxidative stress and dyslipidemia.

**References**
